

## STUDIES ON THE MECHANISM OF ETHANOL-INDUCED HYPOGLYCEMIA \*

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Hypoglycemia after ingestion of ethanolic beverages has been reported (1-4). In some cases (1, 2), hypoglycemia was attributed to the non-ethanol ingredients in such mixtures as "smoke" and "solox" (methyl alcohol, and gasoline and ethyl acetate). Cummins, however, suggested that hypoglycemia might be caused by ethanol itself (3). He described a 6-year-old boy who had convulsions and hypoglycemia after ingestion of gin. Neame and Joubert also ascribed hypoglycemia observed in their patients to ethanol rather than other ingredients (4). They also emphasized the inadequate dietary intake before the ethanolic ingestion and the development of hypoglycemia.

Recently, we studied a chronic alcoholic patient who had several documented episodes of hypoglycemia and minimal evidence of abnormal liver function. Our findings indicated that his hypoglycemia was probably induced by ethanol consumption in combination with a poor dietary intake. Studies were also performed with normal control subjects which indicated that ethanol ingestion after a 2-day fast caused hypoglycemia. During ethanol-induced hypoglycemia, conversion of fructose to glucose was normal, but glycogen synthesis appeared to be inhibited, from the failure of an adequate hyperglycemic response to glucagon. Animal studies suggested that ethanol caused hypoglycemia by interfering with gluconeogenesis as well as glycogen synthesis. Recent studies reported in abstract form have also proposed that the mechanism of ethanol-induced hypoglycemia might be an interference in gluconeogenesis (5).

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### MATERIALS AND METHODS

The patient C.P. was a white, 52-year-old, male plumber. He had a long history of ethanol ingestion averaging approximately one pint of whiskey daily. His dietary habits were variable and he would frequently miss one or two meals a day. His first episode of hypoglycemia came 10 years ago when he suddenly felt dizzy and collapsed after a 24-hour fast. He denied any ethanolic intake just before this episode. He was treated with iv glucose and made a prompt recovery. His next attack came 6 years later under similar circumstances; blood sugar was 28 mg per 100 ml, and he responded rapidly to 25 g iv glucose. One year later, another episode of hypoglycemia with a blood glucose of 28 mg per 100 ml was documented; a 6-hour glucose tolerance test was normal, and a 3-day fast did not cause hypoglycemia. Two years before admission, he was seen at home by his physician for another, similar, but somewhat milder, attack. He gave a history then of heavy alcoholic consumption for the 2 preceding days and no food intake for the 24 hours just before the attack. He was treated with glucose and responded promptly. The recurrence of a similar episode with a blood glucose of 25 mg per 100 ml led to the patient's hospitalization at the Clinical Center of the National Institutes of Health. Physical examination was unremarkable, except for severe, symmetrical, subcutaneous lipomatosis limited to the upper half of the body and bilateral, sensory, peripheral neuropathy of the legs. Hemoglobin was 12 g per 100 ml and the leukocyte count was 3,400 cells per mm<sup>3</sup>. There were 55% polymorphonuclear cells, 37% lymphocytes, 5% eosinophils, and 3% monocytes. Urinalysis was normal; total protein was 6 g per 100 ml, with 2.7 g of albumin, and bromsulfophthalein excretion was 4% in 45 minutes. Serum glutamic-oxaloacetic transaminase was 18 U and alkaline phosphatase was 7.0 King-Armstrong U. Thymol turbidity was 1+, whereas the cephalin flocculation test was negative. Serum bilirubin was 0.7 mg per 100 ml total and cholesterol was 136 mg per 100 ml. Urinary excretion of 17-hydroxycorticosteroids and 17-ketosteroids was 2.6 and 8.0 mg per 24 hours, respectively. Fasting blood glucose varied between 80 and 102 mg per 100 ml. A 6-hour oral glucose tolerance test gave the following results, in milligrams per 100 ml: fasting, 89; ½ hour, 123; 1 hour, 126; 2 hours, 97; 3 hours, 77; 4 hours, 66; 5 hours, 91; and 6 hours, 89.

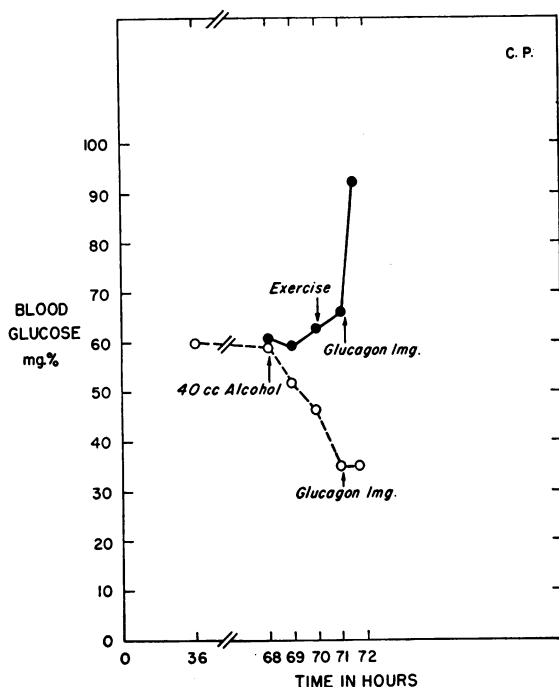


FIG. 1. EFFECT OF 72-HOUR FAST AND ETHANOL ON BLOOD GLUCOSE IN PATIENT C.P. After a 68-hour fast, the patient was given orally 40 ml of ethanol diluted with water or water alone, and blood glucose was determined hourly thereafter. Three hours after ethanol or water, 1 mg im glucagon was administered, and blood glucose was measured 30 minutes later.

Results of the oral administration of *l*-leucine (150 mg per kg) were as follows, in milligrams per 100 ml: fasting blood glucose, 89; 20 minutes, 84; 40 minutes, 79; 60 minutes, 86; 80 minutes, 79; 100 minutes, 84; and 120 minutes, 80. Blood glucose response to 1 g iv tolbutamide (6) was normal and gave the following results, in milligrams per 100 ml: fasting, 80; 30 minutes, 62; 60 minutes, 45; 90 minutes, 63; 120 minutes, 68; 150 minutes, 76; and 180 minutes, 82.

Normal control subjects ranged in age from 18 to 25 and had no evident abnormalities of carbohydrate metabolism.

Absolute or 95% ethanol diluted to a final concentration of 10% with water was given orally after variable periods of fasting. Mild euphoria and drowsiness almost always followed ethanol ingestion. In some studies, 50 g iv fructose was given during 2 minutes 2 hours after ethanol. One mg im glucagon was administered 2 or 3 hours after ethanol.

Male rabbits weighing approximately 2 kg were given 6 ml ethanol per kg body weight diluted with 2 vol of water, or water alone by stomach tube after a 2-day fast. It has been reported that this amount of alcohol produces hypoglycemia in rabbits whose liver glycogen has been depleted by infusions of emulsified lecithin (7). Samples for blood sugar were obtained before administra-

tion of ethanol or water and 1 hour later, since preliminary experiments indicated that then the effect on blood sugar was maximal. After the second blood sugar, the animals were killed by a blow on the head, and the liver was quickly removed. Portions were immediately homogenized in 5% trichloroacetic acid for determination of glycogen, which was precipitated by the addition of 2 vol absolute ethanol. Slices weighing between 100 and 200 mg were prepared with a Stadie-Riggs microtome from the remainder of the liver and incubated in the high potassium buffer of Buchanan, Hastings, and Nesbitt (8) for 90 minutes at 37° C in a Dubnoff metabolic shaker. Glucose concentration was 360 mg per 100 ml and the medium contained 0.25  $\mu$ c (250,000 cpm) of glucose-1-C<sup>14</sup>. The gas phase was 95% O<sub>2</sub> and 5% CO<sub>2</sub>. When liver slices were incubated with pyruvate-2-C<sup>14</sup> (0.25  $\mu$ c and 0.4 mg per 100 ml), no glucose was added to the buffer, and incubation lasted 30 minutes. At the end of incubation, concentration of glycogen and incorporation of radioactivity into the glycogen was determined. In order to correct for possible radioactive contamination of glycogen by the large amount of radioactive substrate added to the incubation flasks, some slices were incubated only momentarily and then homogenized in 5% trichloroacetic acid. Glycogen was precipitated by addition of ethanol and redissolved in water. Radioactivity present in the glycogen in these flasks was considered to be due to trapped substrate and was subtracted from the radioactivity present in glycogen at the end of incubation. This blank radioactivity was less than 5% of that present in glycogen at the end of incubation. To 0.2 ml of the aqueous glycogen solution was added 3.8 ml of hyamine and 10 ml of 0.4% diphenyloxazole in toluene as the phosphor for liquid scintillation counting. Results are expressed as counts per minute in glycogen per 100 mg of liver slice per total incubation period. Oxidation of pyruvate-2-C<sup>14</sup> and glucose-1-C<sup>14</sup> was measured as described previously (9).

Six pairs of isolated livers were perfused as previously described (10). Male rats of the Osborne-Mendel strain weighing between 140 and 150 g were fasted for 18 hours before the experiment. Two perfusions were run simultaneously; one served as a control and ethanol was added to the other at an initial concentration of 0.41%. The ethanol-perfused liver appeared somewhat darker than the control, but there was no obvious difference in its ability to extract oxygen from the perfusing solution. The total amount of perfusing solution was 40 ml. Conversion of fructose to glucose and glycogen was assessed by adding 250 mg of fructose to the perfusing medium 30 minutes after the onset of perfusion. In these experiments, glycogen content of the liver was determined at the end of a 90-minute perfusion. Concentrations of glucose, urea, and amino acids in the perfusing solution were measured at appropriate times in 0.2-ml samples.

Blood glucose was determined with glucose oxidase.<sup>1</sup>

<sup>1</sup> Glucostat, Worthington Biochemical Corp., Freehold, N. J.

TABLE I  
Influence of duration of fast on blood glucose response to ethanol and glucagon

Subject	Duration of fast	Ethanol ingestion†	Blood glucose (in milligrams per 100 ml) minutes after fast*					
			60	120†	180	240	300‡	330
	<i>hours</i>							
P.D.	13	Yes	71	69	69	66	68	119
F.F.	13	Yes	79	71	75	72	66	98
G.S.	13	Yes	78	76	74	69	71	115
C.P.	13	Yes	90	91	85	85	81	
P.D.	44	Yes	62	67	56	52	48	56
P.D.	44	No	65	68	67	66	66	74
C.B.	44	Yes	59	59	58	51	36	40
C.B.	44	No	59	60	56	59	60	70
G.S.	44	Yes	65	64	51	50	49	52
G.S.	44	No	61	61	59	61	63	75
F.F.	44	Yes	58	58	51	45	45	49
F.F.	44	No	65	71	70	64	65	97
M.G.	44	Yes	56	54	45	41	45	52
M.G.	44	No	57	58	56	56	56	68

\* During this 5½-hour period, the subjects continued in the fasting state except for ingestion of ethanol in the appropriate experiments.

† When ethanol was administered, it was given after the 120-minute sample for blood glucose was obtained.

‡ One mg glucagon im was administered after this sample was obtained. See legend for Figures 2 and 3.

Total blood sugars were measured by the method of Somogyi-Nelson (11), and blood fructose was calculated by subtracting the value of glucose obtained with glucose oxidase from the values obtained by copper reduction. Plasma FFA were estimated according to the procedure of Dole (12). Serum urea nitrogen was determined by a commercially designed urease-phenate-hypochlorite method.<sup>2</sup> Amino acid levels were measured in serum protein-free filtrates by the method of Rosen (13). Glycogen was determined by the method of Carroll, Longley, and Roe (14).

## RESULTS

The data in Figure 1 indicate that oral ingestion of 40 ml of ethanol by the patient C.P. after 68 hours of fasting produced hypoglycemia. Administration of 1 ml of glucagon 3 hours later did not increase blood sugar during the next 30 minutes. The patient did not exercise during this experiment. In contrast, fasting for 71 hours with exercise but without ethanol did not cause hypoglycemia, and 1 ml glucagon caused a prompt rise in blood glucose. Hypoglycemia induced by ethanol was not limited to the patient, but was elicited in all of five normal subjects after 44 hours of fasting (Table I). A representative experiment is presented in Figure 2. Hypoglycemia resulted from the ingestion of 35 ml of ethanol and only a small rise in blood glucose was seen after glucagon. Fasting for 48 hours without ethanol

<sup>2</sup> UN-Test, Hyland Laboratory, Los Angeles, Calif.

did not cause hypoglycemia, and there was a somewhat greater increase in blood glucose after glucagon. Three subjects as well as patient C.P. did

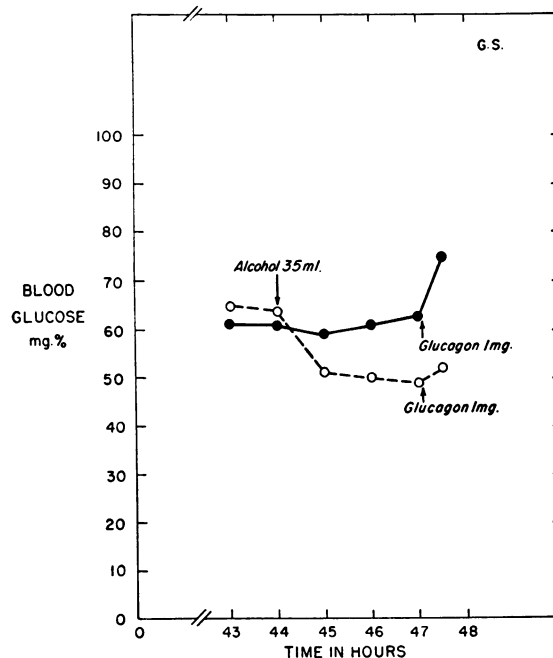


FIG. 2. EFFECT OF 48-HOUR FAST AND ETHANOL ON BLOOD GLUCOSE IN NORMAL SUBJECT. After a 44-hour fast, the subject was given 35 ml of ethanol diluted with water or water alone and blood glucose was determined hourly thereafter. One mg glucagon was given 3 hours after ethanol or water, and blood glucose was measured 30 minutes later.

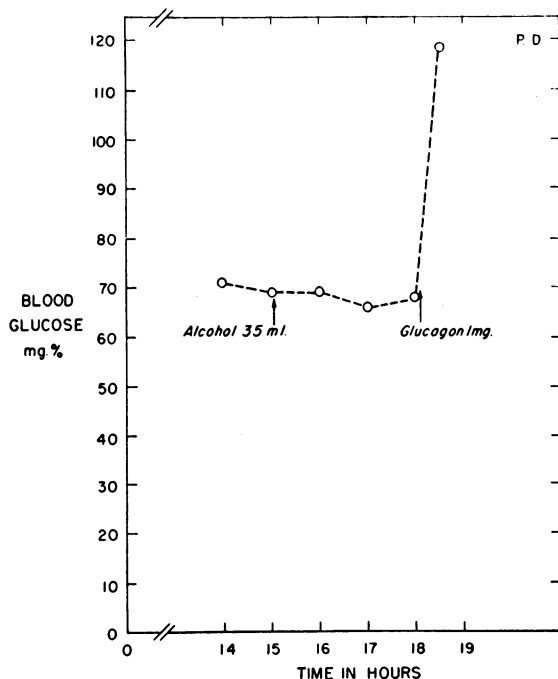


FIG. 3. FAILURE OF ETHANOL TO CAUSE HYPOGLYCEMIA OR INHIBIT HYPERGLYCEMIC EFFECT OF GLUCAGON AFTER AN OVERNIGHT 15-HOUR FAST.

not develop hypoglycemia when 35 to 50 ml of ethanol was administered after an overnight fast, and there was no inhibition of the hyperglycemic response to 1 mg im glucagon (Table I). A representative experiment is shown in Figure 3. These studies indicate that the failure of glucagon to raise blood sugar during ethanol-induced hypoglycemia is not due to an inhibition of glucagon action on liver glycogen, but would be consistent with marked depletion of liver glycogen at the time of hypoglycemia. Ethanol after a 24-hour fast did not produce hypoglycemia, whereas it always did so after a 44-hour fast.

In one subject, plasma FFA were also measured with blood glucose after ethanol and a 44-hour fast (Figure 4). During ethanol-induced hypoglycemia, there was a progressive rise in plasma FFA, suggesting that the hypoglycemia was not primarily insulin-mediated (12). Similar conclusions were proposed by Freinkel, Singer, Siebert, and Anderson on the basis of insulin assay and the absence of any increase in peripheral glucose utilization during ethanol-induced hypoglycemia (5). When a similar degree of hypoglycemia was produced with 10 U of regular insulin

given subcutaneously, an initial fall in plasma FFA was observed that corresponded to the nadir of blood glucose. As blood glucose returned to normal, there was a secondary rise in FFA. Fasting for 48 hours alone was not associated with hypoglycemia, nor with a progressive change in plasma FFA during the last 5 hours of the fast.

The effect of ethanol on conversion of fructose to glucose and glycogen was studied in four control subjects and the patient C.P. with identical results (Table II). The data from one of these studies is presented in Figure 5. Administration of fructose during ethanol-induced hypoglycemia caused an increase in blood glucose greater than that produced by the same amount of fructose without antecedent ethanol. Thus, conversion of fructose to glucose was not inhibited by ethanol. The synthesis of glycogen from fructose, however, appeared to be decreased by ethanol, since there was only a small further rise in blood glucose after glucagon in the ethanol experiments as compared with the control experiments. Prior administration of ethanol did not appear to influence the rate of disappearance of fructose from the blood (Table III). Similar inhibition by ethanol of glycogen synthesis from fructose was also demon-

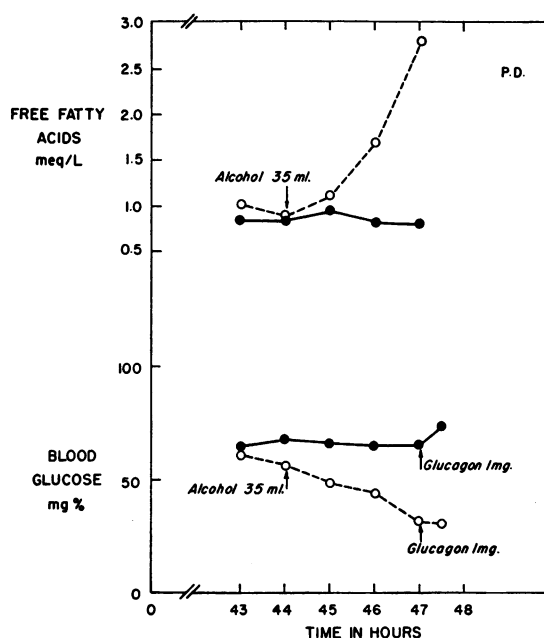


FIG. 4. RESPONSE OF PLASMA FREE FATTY ACIDS TO ETHANOL-INDUCED HYPOGLYCEMIA. Experimental details are like those for Figure 2.

TABLE II  
Effect of ethanol on conversion of fructose to glucose and glycogen

Subject	Ethanol ingestion*	Blood glucose (in milligrams per 100 ml) at hours of fast							
		43	44*	45	46†	46.33	46.67	47‡	47.5
M.G.	Yes	56	54	45	41	45	95	95	131
M.G.	No	57	58	56	56	56	83	76	165
C.B.	Yes	60	58	47	36	37	89	102	126
C.B.	No	59	60	56	59	75	77	75	168
G.S.	Yes	63	56	45	41	43	81	87	108
G.S.	No	59	58	60	62	72	82	77	127
F.F.	Yes	55	61	55	46	54	89	90	100
F.F.	No	64	66	64	65	65	87	79	142
C.P.	Yes	55	54	45	38	42	60	68	81
C.P.	No	58	60	57	61	66	80	77	130

\* When ethanol was administered, amounts equivalent to 35 to 50 ml were given after the 44-hour sample for blood glucose was obtained.

† Fifty g iv fructose was administered during 2 minutes after this sample was obtained.

‡ One mg im glucagon was administered after this sample was obtained. See legend for Figure 5.

strated in two sets of perfusion studies of isolated rat liver (Table IV). During the first 30 minutes of the perfusion, there was an increase in glucose concentration of the perfusion medium representing both glycogen breakdown and gluconeogenesis. After addition of fructose to the perfusate, increases in glucose concentration in the media were noted in both control and ethanol-perfused livers. In the presence of the higher concentration of ethanol in the perfusion (1.6%), conversion of fructose to glucose was not so rapid as in the control liver. The similarity, however, of these increases when the initial ethanol concen-

tration was 0.41% indicated that conversion of fructose to glucose was not inhibited by this amount of ethanol. When glycogen content of the livers was measured at the end of perfusions, considerably less glycogen was present in ethanol-perfused livers than in the controls.

TABLE III  
Failure of ethanol to influence rate of fructose disappearance\*

Subject	Ethanol ingestion	Blood fructose minutes after iv fructose		
		20	40	60
		mg/100 ml		
M.G.	No	135	52	17
	Yes	143	59	25
C.B.	No	108	37	14
	Yes	134	60	29
G.S.	No	76	26	8
	Yes	115	46	18
F.F.	No	90	37	10
	Yes	84	25	11
C.P.	No	105	51	33
	Yes	92	39	20

\* Fifty g iv fructose was administered during 2 minutes, and blood samples were obtained at 20-minute intervals for the next 60 minutes. Fructose was determined as the difference between the total reducing substances measured by Somogyi-Nelson method (11), and the blood glucose values were obtained with glucose oxidase.

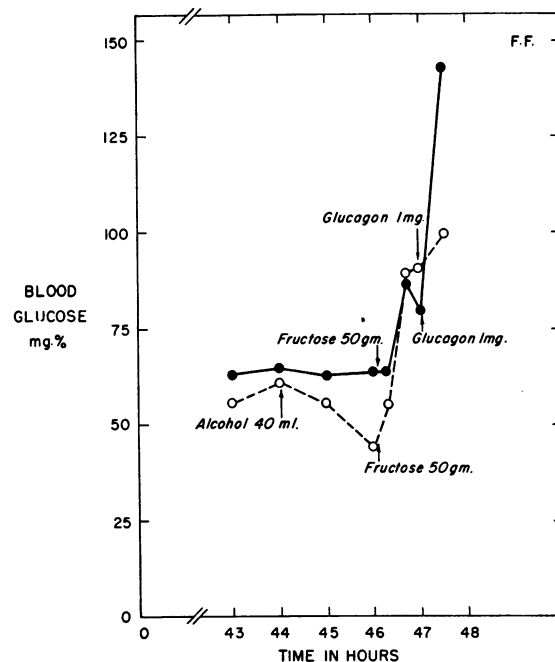


FIG. 5. EFFECT OF ETHANOL ON CONVERSION OF FRUCTOSE TO GLUCOSE AND GLYCOGEN. After a 44-hour fast, the subject was given 40 ml of ethanol diluted with water or water alone, and blood sugar was measured for the next 3½ hours. Two hours after ethanol or water, 50 g iv fructose was administered and 1 hour later, 1 mg im glucagon.

TABLE IV  
*Effect of ethanol on the conversion of fructose to glucose and glycogen by the isolated perfused liver\**

Experiment	Ethanol added to perfusion	Glucose concentration (in milligrams per 100 ml) at minutes of perfusion						Glycogen content at end of perfusion mg/g
		0	30	45	60	75	90	
1	No	39	85	137	211	303	345	3.5
	Yes	32	56	91	147	206	257	0.4
2	No	68	117	146	209	274	345	3.9
	Yes	69	100	143	207	286	347	1.5

\* In experiment 1, ethanol at an initial concentration of 1.6% was added to the perfusing medium of one liver at zero time. In experiment 2, initial ethanol concentration was 0.41%. After 30 minutes of perfusion, 250 mg of fructose was added to the perfusion medium of the livers. At the end of perfusion, glycogen content of the liver was measured.

Further confirmation of ethanol-induced inhibition of glycogen synthesis was obtained from studies with rabbits treated with ethanol (Table V). During the 1 hour after ethanol administration, blood glucose decreased, whereas it increased in the rabbits given water. The difference in response of these two groups is statistically significant. The rise in blood glucose probably reflects epinephrine discharge concomitant with intubation of unanesthetized, restrained animals. One hour after administration of either ethanol or water, the animals were killed and liver glycogen content was determined. There was significantly less glycogen in the livers of the rabbits treated with ethanol than in the controls (Table V). These changes in liver glycogen are consistent with those reported earlier by Forbes and Duncan (15). Since there was an actual decline in blood glucose of ethanol-treated rabbits, it is difficult to attribute the decrease in liver glycogen to augmented glycogenolysis. Liver slices from ethanol-treated rabbits incorporated considerably less radioactive glucose-1-C<sup>14</sup> into glycogen during a 90-minute incubation

than did those from control rabbits. The actual glycogen content of the slices changed very little during incubation (Table V). Liver slices from rabbits given ethanol also oxidized less than 50% as much glucose-1-C<sup>14</sup> as did slices from control animals (Table VI). When pyruvate-2-C<sup>14</sup> was the substrate, oxidation to C<sup>14</sup>O<sub>2</sub> was less than 25% of control values, and incorporation of label from pyruvate into glycogen was about 50% of that in slices from control rabbits.

In addition to its inhibition of glycogen synthesis, ethanol also interfered with gluconeogenesis as measured by urea production by isolated rat liver in four sets of experiments (Table VII). Data from a representative experiment is given in Figure 6. In the ethanol-perfused liver, glucose production was decreased approximately 50%, as was urea production. Amino acid production by both control and ethanol-perfused livers was approximately the same, suggesting that the block in gluconeogenesis is probably in the deamination of amino acids rather than the breakdown of protein.

TABLE V  
*Effect of ethanol in vivo on blood glucose and in vitro glycogen synthesis\**

Treatment	No. of rabbits	Blood sugar change in 1 hour mg/100 ml	Liver glycogen		Radioactivity incorporated into glycogen from glucose cpm/100 mg liver
			Before incubation mg/g	After incubation mg/g	
Water	5	+ 25 ± 6†	11.9 ± 1.7†	10.4 ± 0.3†	15,600 ± 2,100†
Ethanol	5	- 9 ± 5	4.4 ± 0.3	3.5 ± 1.1	7,400 ± 1,600

\* Rabbits were tube-fed either ethanol or water, and change in blood sugar over the next 60 minutes was measured. The animals were then sacrificed, and glycogen content of the liver was determined. Liver slices were then incubated for 90 minutes and the amount of glycogen and incorporation of radioactive glucose-1-C<sup>14</sup> into glycogen ascertained. Glucose concentration was 360 mg per 100 ml.

† Standard error of the mean.

TABLE VI  
Effect of ethanol on glucose-1-C<sup>14</sup> and pyruvate-2-C<sup>14</sup> oxidation to C<sup>14</sup>O<sub>2</sub> by rabbit liver slices\*

Treatment	No. of rabbits	C <sup>14</sup> O <sub>2</sub> derived from		Radio-activity incorporated into glycogen from pyruvate
		Glucose-1-C <sup>14</sup>	Pyruvate-2-C <sup>14</sup>	
Water	2	cpm/g	cpm/g	cpm/100 mg liver
		15,250	122,400	3,655
Ethanol	2	5,105	23,910	1,495
		6,370	24,475	1,083

\* Rabbits were tube-fed either ethanol or water 1 hour before sacrifice. Liver slices were incubated in a glucose concentration of 360 mg per 100 ml and 250,000 cpm glucose-1-C<sup>14</sup>, or in a pyruvate concentration of 0.4 mg per 100 ml and 250,000 cpm pyruvate-2-C<sup>14</sup>.

## DISCUSSION

These studies indicate that ethanol ingestion can produce hypoglycemia and that previously reported cases (1-4) were probably due to ethanol rather than some other nonethanol toxin. Prior poor nutrition is evidently important in the genesis of ethanol hypoglycemia; 35 to 50 ml of ethanol did not cause hypoglycemia when it was administered after an overnight fast, but did so after a 44-hour fast. This observation confirms the earlier findings of Tennent (16) and Lieber and co-workers (17), who also noted no effect of ethanol on blood glucose after an overnight fast. As a consequence of inadequate dietary intake, liver glycogen is probably depleted and this would also contribute to hypoglycemia. The importance of glycogen depletion is suggested by earlier studies

TABLE VII  
Effect of ethanol on glucose, urea, and amino acid production by isolated perfused liver

Experiment	Ethanol added to perfusion	Net amino acids produced	Net urea-N produced	Increase in perfusion glucose concentration
1	Yes	60	70	82
	No	44	193	160
2	Yes	75	124	90
	No	90	292	208
3	Yes	67	172	49
	No	61	321	81
4	Yes	61	279	48
	No	61	321	71

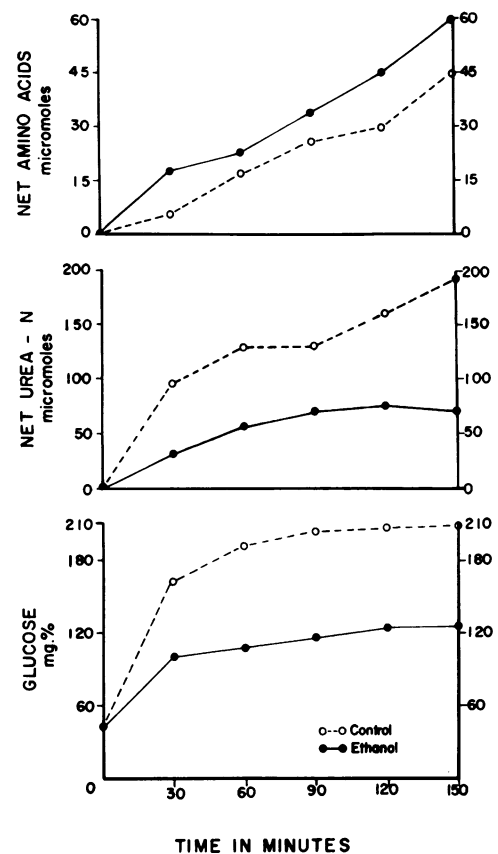


FIG. 6. EFFECT OF ETHANOL ON GLUCOSE, UREA, AND AMINO ACID PRODUCTION BY ISOLATED PERFUSED LIVER. Ethanol at an initial concentration of 0.41% was added to the perfusion medium of one liver at zero time. At 30-minute intervals for the next 2½ hours, samples of perfusion medium were removed for determination of glucose, urea, and amino acids.

in animals in which hypoglycemia was produced only in those whose livers were devoid of glycogen (7, 16). This is consistent with the observations of Neame and Joubert, who reported liver glycogen absent from biopsy specimens obtained on their patients during hypoglycemia (4). Indirect evidence obtained during the present studies also suggests that diminished liver glycogen is present during ethanol-induced hypoglycemia, since administration of glucagon resulted in very little, if any, increase in blood sugar. It seems unlikely that ethanol directly inhibits the effect of glucagon, since there was a brisk rise in blood glucose when glucagon was given after ethanol and an overnight fast (Table I and Figure 3). It is possible that the presence of adequate liver glycogen

after only an overnight fast may account for the failure of ethanol to induce hypoglycemia at this time. The apparently normal response to glucagon in patient C.P. after 71 hours of fasting and exercise (Figure 1) is probably related to increased lactate as a result of exercise and its subsequent conversion to liver glycogen. Blood glucose response to glucagon after 47 hours of fasting without ethanol was small, further suggesting that liver glycogen was decreased under these conditions (Table I, Figures 2 and 4).

The possibility should be considered that the consequences of ethanol on carbohydrate metabolism represent generalized toxic effects on the liver. Neame and Joubert postulated that poor dietary intake makes the liver especially vulnerable to toxic substances such as ethanol (4). Liver biopsy specimens from their patients, however, revealed little evidence of severe hepatic disease, although most of their patients had elevations of serum transaminase. Freinkel and associates also reported normal or only minimally abnormal hepatic histology in their patients who were biopsied (5). In the present studies, serum transaminase values did not exceed the upper limits of normal during or after hypoglycemia.<sup>3</sup> None of the control subjects had any abnormalities in hepatic function. Furthermore, the patient C.P. had only minimally abnormal liver function at a time when ethanol caused hypoglycemia. Ethanol does not produce such a generalized toxic effect on the liver, so that the disappearance of fructose and its conversion to glucose were impaired (Table III and Figure 5).

These studies indicate that in the presence of ethanol there is depressed *a*) urea production in the perfused rat liver, *b*) glycogen synthesis as measured *in vivo* and *in vitro*, and *c*) *in vitro* oxidation of added glucose and pyruvate by the liver. Decreased production of urea by the liver perfused with ethanol would impair gluconeogenesis and thus contribute to the diminished glucose release by this preparation (Table VII and Figure 6). Whereas glucose released during the first 30 minutes of perfusion represents both gluconeogenesis and glycogenolysis, that during the subsequent period is a manifestation almost solely of gluconeogenesis, since glycogen present at the beginning of

perfusion is no longer detectable after 30 minutes.<sup>4</sup> Since less glucose was released by the ethanol-perfused liver throughout the entire experiment, it suggests that the impaired urea formation is associated with diminished gluconeogenesis. In each individual experiment, however, there was usually no good correlation between the amount of urea and glucose produced by either the control or the ethanol-perfused liver. The defect in gluconeogenesis appeared to involve the oxidative deamination of amino acids and not the formation of amino acids from protein breakdown, since the amino acid production by control and ethanol-perfused livers was not significantly different (Table VII). The final steps in gluconeogenesis were not inhibited by ethanol, since fructose was converted to glucose normally by ethanol-perfused liver as well as by control subjects during ethanol-induced hypoglycemia. Ethanol inhibition of gluconeogenesis would be consistent with the previous findings that hepatic glucose production is depressed both *in vivo* (18) and *in vitro* (19). In the latter study, decreased glucose output was attributed to the lower glycogen levels present in the livers of ethanol-treated rats. The mechanism by which ethanol inhibits urea production is not known. One could speculate that it is a consequence of changes in DPN and DPNH levels in the liver, resulting from the metabolism of alcohol (20). Effects of changes in these nucleotides as a result of ethanol oxidation on general metabolic processes (21) and galactose oxidation (22, 23) in the liver have been postulated. The deamination of many amino acids involves the transfer of the amino group to  $\alpha$ -ketoglutarate and the formation of glutamate. Glutamate is then oxidatively deaminated by the enzyme glutamic dehydrogenase. This enzyme has been shown to be sensitive to the levels of DPN and DPNH *in vitro* (24). DPNH favors the dissociation of the enzyme into smaller units that are no longer active with glutamic acid.

The postulated block in glycogen synthesis is based on both *in vivo* and *in vitro* observations. Ethanol-perfused liver converted fructose to glucose as well as control liver, but at the end of the experiment the concentration of glycogen was much greater in the control. Since glucose 6-phos-

<sup>3</sup> Unpublished observations.

<sup>4</sup> G. E. Mortimore, unpublished experiments.



phate is a common intermediate in the metabolism of fructose to both glucose and glycogen, it suggests that inhibition of glycogen synthesis occurs after the formation of glucose 6-phosphate. The diminished blood glucose response to glucagon in humans after ethanol and fructose as compared to the response after fructose alone also points to an impairment in glycogen synthesis, since ethanol did not interfere with either the disappearance of fructose (Table III) or its conversion to glucose (Table II and Figure 5). Furthermore, administration of ethanol to rabbits resulted in decreased liver glycogen 1 hour later. It would be unlikely that this represented increased glycogen breakdown in these animals, since there was an increase in the blood glucose of the control rabbits, as compared to no change or even a fall in the blood glucose of ethanol-treated rabbits. The failure of ethanol to produce more consistent hypoglycemia in rabbits might be due to persistence of some liver glycogen even after a 2-day fast. The relationship between liver glycogen and the effect of ethanol on blood glucose was studied by Tennent (16). Matunaga depleted the liver glycogen of animals with emulsified lecithin before inducing hypoglycemia with ethanol (7). Decreased incorporation *in vitro* of glucose-1-C<sup>14</sup> and pyruvate-2-C<sup>14</sup> into glycogen by liver slices from ethanol-treated rabbits is also consistent with inhibition of glycogen synthesis, although it might also reflect dilution of the labeled substrate by unlabeled acetate resulting from ethanol oxidation.

Glucose-1-C<sup>14</sup> and pyruvate-2-C<sup>14</sup> oxidation by liver slices from ethanol-treated rats was also decreased. Lieber and Schmid reported that ethanol inhibited C<sup>14</sup>O<sub>2</sub> production from labeled acetate (25). These findings could result from dilution of the labeled substrate with unlabeled acetate from ethanol oxidation, or perhaps be a consequence of changes in DPN and DPNH.

The relationship of these effects of ethanol to the production of hypoglycemia must now be considered. Hypoglycemia did not appear to be insulin-mediated, since it was not associated with a fall in plasma FFA. Lieber and co-workers found that within 30 minutes ethanol caused a transient fall in plasma FFA, but subsequently there was a return to normal (17). There were, however, no changes in the blood glucose associated with the fall in plasma FFA, indicating that insulin was

probably not involved. Freinkel and associates did not find evidence of increased plasma insulin or peripheral glucose metabolism during ethanol-induced hypoglycemia (5). Furthermore, ethanol did not inhibit the glycogenolytic action of glucagon and cause hypoglycemia by this mechanism. In the postabsorptive state, hepatic release of glucose is of primary importance for the maintenance of normal blood glucose. Initially, this is accomplished by breakdown of liver glycogen and subsequently, by the process of gluconeogenesis. Since a 2-day fast with its attendant decrease in liver glycogen is essential for the production of ethanol-hypoglycemia, it would seem that inhibition of gluconeogenesis is more important. If there is adequate liver glycogen, then blood glucose can be maintained by its breakdown, and inhibition of gluconeogenesis would not cause hypoglycemia. Thus, whether ethanol causes hypoglycemia clinically would seem to depend on whether there is sufficient liver glycogen to maintain blood glucose. Ethanol did not produce hypoglycemia after an overnight fast, since liver glycogen stores were probably adequate to maintain blood glucose.

#### SUMMARY

Hypoglycemia has been consistently produced in humans by the ingestion of 35 to 50 ml of ethanol after a 2-day fast. Similar amounts of ethanol were ineffective after an overnight fast and did not interfere with the hyperglycemic response to glucagon. The failure of glucagon to increase blood glucose during ethanol-induced hypoglycemia would be consistent with an absence of liver glycogen. Hypoglycemia was associated with a rise in plasma free fatty acids, suggesting that it was not insulin-mediated. During hypoglycemia, intravenously administered fructose was normally converted to glucose, but there was some inhibition of its conversion to glycogen. Studies using perfused rat livers and liver slices from ethanol-treated rabbits also demonstrated decreased glycogen synthesis. Besides inhibiting glycogen synthesis, ethanol also interfered with glucose and urea formation in the isolated, perfused rat liver, but not with amino acid mobilization, suggesting a block in oxidative deamination of amino acids. Inhibition of gluconeogenesis is probably primarily responsible for hypoglycemia, in conjunction with depletion of liver glycogen.

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